

Atty Docket No. 02307O-077630US

PTO FAX NO.: (703) 746-5032

ATTENTION: Examiner Cynthia Collins  
TELEPHONE NO.:

Group Art Unit 1638

**OFFICIAL COMMUNICATION  
FOR THE PERSONAL ATTENTION OF  
EXAMINER Cynthia Collins**

**CERTIFICATION OF FACSIMILE TRANSMISSION**

I hereby certify that the following document(s) in re Application of John Harada, Application No. 09/516,052, filed March 1, 2000 for LEAFY COTYLEDON1 GENES AND THEIR USES is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

Document(s) Attached

1. Declaration of John J. Harada
2. CV of John J. Harada

Number of pages being transmitted, including this page: 27

Dated: July 25, 2002

*Joy M. Marshall*  
Joy M. Marshall

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SF 1369242 v1

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Assistant Commissioner for Patents  
Washington, D.C. 20231

On May 6, 2002

TOWNSEND and TOWNSEND and CREW LLP

By: Jay M. Marshall

PATENT

Attorney Docket No.: 02307O-077630US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

John Harada, *et al.*

Application No.: 09/516,052

Filed: March 1, 2000

For: LEAFY COTYLEDON1 GENES  
AND THEIR USES

Examiner: Cynthia Collins

Art Unit: 1638

DECLARATION UNDER 37 C.F.R. §  
1.132 OF JOHN J. HARADA, PH.D.

Assistant Commissioner for Patents  
Washington, D.C. 20231

I, John J. Harada, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I graduated from University of California, Los Angeles with a bachelor of science degree in biochemistry in 1975. In 1981, I graduated from University of Washington, Seattle with a Ph.D. degree in biochemistry. I have published more than 46

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scientific papers on the subject of plant biology and plant reproductive and seed development.  
A copy of my curriculum vitae is attached hereto as Exhibit A.

3. I am currently a professor at the University of California, Davis. I have been in this position for 17 years.

4. I have read and am familiar with the contents of the above-referenced patent application. I understand that the present invention provides compositions and methods for modulating embryo development in plants. The application provides at least three representative sequences with sequence homology to the B domain, a sequence originally identified in yeast HAP3 proteins. Based on sequence homology and functional experiments, the application demonstrates that polypeptides comprising homology to the B domain of the *Arabidopsis LEC1* sequence (SEQ ID NO:2) can modulate embryo development when introduced into plants.

4. I have read the Office Action (Paper No. 13) in the present case dated December 4, 2001. It is my understanding that the Examiner is concerned that the scope of the claimed subject matter is not supported by the teaching of the present application. In particular, the Examiner states that the application does not "set forth what specific structural or physical features are required in the claimed isolated nucleic acids." See Paper No. 13, page 5. The Examiner also appears to question whether other polypeptides within the scope of the claims would have the activity of the *Arabidopsis LEC1* protein (SEQ ID NO:2). As demonstrated below, as of the filing date, the application provided sufficient information for those of skill in the art to identify and generate polypeptides that were 80% identical to SEQ ID NO:2, which when introduced into plants, modulate embryo development. The following data is provided to demonstrate that polypeptides that are at least 80% identical to the B domain of SEQ ID NO:2 do in fact modulate embryo development when introduced into plants. Indeed, the data shows that sequences with as little as 68% identity to the B domain can modulate embryo activity.

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5. To identify regions of the LEC1 protein that are responsible for its unique activity in promoting embryo development, we developed an assay that would allow us to assess the ability of a transgene to suppress the *lec1* mutation. In this assay, the gene of interest is fused with DNA fragments containing regulatory sequences that control *LEC1* gene expression. Specifically, the coding region of the gene of interest is fused with the 1997 bp region upstream of the *LEC1* translation initiation codon and with the 774 bp region downstream of the *LEC1* translation termination codon. These sequences allow for expression corresponding to the native *LEC1* gene. The chimeric gene is then transferred into *Arabidopsis lec1-1* null mutants using the floral dip method (Bechtold *et al.*, *C.R. Acad. Sci. Paris* 316, 1194-1199 (1993)). *lec1-1* mutant embryos are intolerant of desiccation, and dried *lec1* mutant seeds do not germinate because the embryos are not viable. See, e.g., Meinke, *D.W. Science* 258, 1647-1650 (1992) and West, M.A.L., *et al. Plant Cell* 6, 1731-1745 (1994). If transformation of *lec1-1* mutants with a polynucleotide results in the production of viable progeny, then the transgene suppresses the *lec1* mutation, and therefore modulates embryo development. The final step of the assay is to germinate seeds, determine if viable seedlings are produced, and confirm the genotype of the progeny. The results of the assay can be compared to a positive control, i.e., expression of *LEC1* itself.

6. We analyzed the LEC1-LIKE (L1L) protein (SEQ ID NO: 20 in the present application) to determine if it possesses *LEC1* function. L1L is an *Arabidopsis* HAP3 protein. HAP3 subunits can be divided functionally into three domains: an amino-terminal A domain, a central B domain that is conserved among eukaryotic organisms, and a carboxyl-terminal C domain. Analysis using the Gap program of the Wisconsin GCG Package based on the alignment method of Needleman and Wunsch (Needleman and Wunsch, *J. Mol. Biol.* 48, 443-453 (1970)) showed that *LEC1* and L1L display no significant sequence identity in the A or C domains. See, Figures 1 and 2. In contrast, as shown in Figure 3, the B domains of *LEC1* and L1L exhibit 83.3% sequence identity.

7. Using the assay described above, we showed that L1L possesses *LEC1* activity. *lec1-1* mutant plants transformed by the floral dip method with constructs containing the *LEC1* and L1L genes yielded 0.61% and 0.65% viable seedlings, respectively. Thus, L1L,

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which shares 83.3% amino acid sequence identity with the central B domain of LEC1, complements the *lec1* mutation as well as LEC1 itself. Because LEC1 and L1L do not share significant sequence identity in the A or C domains, this result indicates that the B domain mediates LEC1 activity.

8. We also showed that a different *Arabidopsis* HAP3 homolog, At4g14540, could be modified to possess LEC1 function. We changed a single amino acid at position 28 in the B domain of At4g14540 from lysine (K) to aspartic acid (D). The aspartic acid residue is present at the corresponding position in LEC1 and L1L but not in all other *Arabidopsis* HAP3 homologs. As shown in Figure 4, this modified K28D At4g14540 B domain shares 67.8% amino acid sequence identity with LEC1. Using the genetic suppression assay, we showed that a HAP3 protein with the modified K28D At4g14540 B domain produced 0.23% viable seedlings. In contrast, a HAP3 protein with an unaltered At4g14540 B domain only minimally suppressed the *lec1-1* mutation, generating 0.028% viable seedlings. Thus, the modified K28D At4g14540 HAP3 protein, with only 67.8% amino acid sequence identity with the LEC1 B domain, possesses LEC1 activity. These results show that a polypeptide comprising a subsequence about 68% identical to the B domain of *Arabidopsis* LEC1 can function in place of LEC1.

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9. These results confirm the assertions in the present application that polypeptides comprising a subsequence similar to the B domain of SEQ ID NO:2 of the present application can modulate embryo development when introduced into plants. It is my scientific opinion that the specification provides sufficient sequence information to allow those of skill in the art to identify and generate polypeptides comprising a subsequences at least 80% identical to the B domain of SEQ ID NO:2. The data presented in this declaration further confirms that such sequences modulate embryo development when introduced into a plant. Indeed, the data presented here demonstrates that sequences with even lower identity than 80% retain LEC1 activity. Therefore, there can be no question that those of skill in the art would have recognized that the specification provides sufficient structural features to adequately describe active polypeptides and polynucleotides encoding such polypeptides.

Date: 5/31/02

By: J. Harada

John J. Harada, Ph.D.

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FIGURE 1. Amino acid sequence alignment of LEC1 and L1L A domains.

LEC1\_A\_Domain\_Only Length: 27 March 4, 2002 15:34 Type: P Check:  
 9027 ..

MTSSVIVAGA GDKNNNGIVVQ QQPPCVA

L1L\_A\_Domain\_only Length: 56 March 4, 2002 15:33 Type: P Check:  
 1292 ..

MERGGFHYGR KLSVNNTTPS PPGLAANFLM AEGSMRPPEF NQPNKTSNGG  
 EEECTV

GAP of: L1L\_A\_Domain\_only check: 1292 from: 1 to: 56  
 to: LEC1\_A\_Domain\_Only check: 9027 from: 1 to: 27

Symbol comparison table: blosum62.cmp CompCheck: 1102  
 BLOSUM62 amino acid substitution matrix.  
 Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid  
 substitution matrices from protein blocks. Proc. Natl.

Acad.

Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778  
 Length Weight: 2 Average Mismatch: -2.248

Quality: 2 Length: 80  
 Ratio: 0.074 Gaps: 0

Percent Similarity: 33.333 Percent Identity: 33.333

Match display thresholds for the alignment(s):

| = IDENTITY  
 : = 2  
 . = 1

L1L\_A\_Domain\_only x LEC1\_A\_Domain\_Only April 15, 2002 14:27 ..

51 EEECTV..... 56  
 |  
 1 ...MTSSVIVAGAGDKNNNGIVVQQQPPCVA 27

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FIGURE 2. Amino acid sequence alignment of LEC1 and L1L C domains.

LEC1\_C\_Domain\_Only Length: 90 March 4, 2002 15:35 Type: P Check:  
 817 ..

EIETDRGSAL RGEPPSLRQT YGGNGIGFHG PSHGLPPPGP YGYGMLDQSM  
 VMGGGRYYQN GSSGQDESSV GGGSSSSING MPAFDHYGQY

L1L\_C\_Domain\_Only Length: 88 March 4, 2002 15:34 Type: P Check:  
 6801 ..

ELEGERGVSC SAGSVSMTNG LVVKRPNGTM TEYGAYGPVP GIHMAQYHYR  
 HQNGFVFSGN EPNSKMSGSS SGASGARVEV FPTQQHKY

GAP of: L1L\_C\_Domain\_Only check: 6801 from: 1 to: 88  
 to: LEC1\_C\_Domain\_Only check: 817 from: 1 to: 90

Symbol comparison table: blosum62.cmp CompCheck: 1102  
 BLOSUM62 amino acid substitution matrix.

Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid  
 substitution matrices from protein blocks. Proc. Natl.

Acad.

Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778  
 Length Weight: 2 Average Mismatch: -2.248

Quality: 33 Length: 99  
 Ratio: 0.375 Gaps: 6  
 Percent Similarity: 43.038 Percent Identity: 34.177

Match display thresholds for the alignment(s):

| = IDENTITY  
 : = 2  
 . = 1

L1L\_C\_Domain\_Only x LEC1\_C\_Domain\_Only April 15, 2002 14:34 ..

1 ELEGERGVSCSAGSVSMT....NGLVVKRPNGTMTEYGAYG...PVPGI 42  
 | : | : || . | : || : | . : | || .  
 1 EIETDRGSALRGEPPSLRQTYGGNGIGFHG PSHGLPPPGP YGYGMLDQSM 50  
 43 HMAQYHYRHQNGFVFSGNEPNSKMSGSSGASGARVEV FPTQQHKY... 88  
 | : || . | : . | || . | . | | |  
 51 VMGGGRYYQNG..SSGQDESSVGGSSSSING....MPAFDH.YGQY 90

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FIGURE 3. Amino acid sequence alignment of LEC1 and LIL B domains.

LEC1\_B\_Domain\_Only Length: 90 March 4, 2002 15:35 Type: P Check:  
 9031 ..

REQDQYMPIA NVIRIMRKTL PSHAKISDDA KETIQECVSE YISFVTGEAN  
 ERCQREQRKT ITAEDILWAM SKLGFDNYVD PLTVFINRYR

LIL\_B\_Domain\_Only Length: 90 March 4, 2002 15:33 Type: P Check:  
 8221 ..

REQDRFMPPIA NVIRIMRRIL PAHAKISDDS KETIQECVSE YISFITGEAN  
 ERCQREQRKT ITAEDVLWAM SKLGFDYYIE PLTLYLHRYR

GAP of: LIL\_B\_Domain\_Only check: 8221 from: 1 to: 90  
 to: LEC1\_B\_Domain\_Only check: 9031 from: 1 to: 90

Symbol comparison table: blosum62.cmp CompCheck: 1102  
 BLOSUM62 amino acid substitution matrix.

Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid  
 substitution matrices from protein blocks. Proc. Natl.

Acad.

Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778  
 Length Weight: 2 Average Mismatch: -2.248

Quality: 419 Length: 90  
 Ratio: 4.656 Gaps: 0

Percent Similarity: 92.222 Percent Identity: 83.333

Match display thresholds for the alignment(s):

| = IDENTITY

: = 2

. = 1

LIL\_B\_Domain\_Only x LEC1\_B\_Domain\_Only April 15, 2002 14:32 ..

1 REQDRFMPPIANVIRIMRRILPAHAKISDDS KETIQECVSE YISFITGEAN 50

||||.:.|||||:||.|||||.|||||:|||||:|||||

1 REQDQYMPIANVIRIMRKTLPSHAKISDDAKETIQECVSE YISFVTGEAN 50

51 ERCQREQRKTITAEDVLWAMSKLGFDYYIEPLTLYLHRYR 90

|||||:|||||.|||||.||:||||.:.|||||

51 ERCQREQRKTITAEDILWAMSKLGFDNYVDPLTVFINRYR 90

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FIGURE 4. Amino acid sequence alignment of B domains of LEC1 and At4g14540 (247D4) with a site directed change of B domain residue 28 from K (lysine) to D (aspartic acid).

LEC1\_B\_Domain\_Only Length: 90 March 4, 2002 15:35 Type: P Check:  
 9031 ..

REQDQYMPIA NVIRIMRKTL PSHAKISDDA KETIQECVSE YISFVTGEAN  
 ERCQREQRKT ITAEDILWAM SKLGFDNYVD PLTVFINRYR

AHAP3-247D4 (At4g14540) B domain with K to D change

REQDRFLPIA NVSRIMKKAL PANAKISDDA KETVQECVSE FISFITGEAS  
 DKCQREKRKT INGDDLLWAM TTLGFEDYVE PLKVYLQKVR

GAP of: 247D4\_Bdom check: 7153 from: 1 to: 90  
 to: LEC1pepshort check: 9031 from: 1 to: 90

Symbol comparison table: blosum62.cmp CompCheck: 1102  
 BLOSUM62 amino acid substitution matrix.  
 Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid  
 substitution matrices from protein blocks. Proc. Natl.

Acad.

Sci. USA 89: 10915-10919.

Gap Weight: 8 Average Match: 2.778  
 Length Weight: 2 Average Mismatch: -2.248

Quality: 355 Length: 90  
 Ratio: 3.944 Gaps: 0  
 Percent Similarity: 84.444 Percent Identity: 67.778

Match display thresholds for the alignment(s):

| = IDENTITY  
 : = 2  
 . = 1

247D4\_Bdom x LEC1pepshort April 29, 2002 16:36 ..

1 REQDRFLPIANVSRRIMKKALPANAKISDDAKETVQECVSEFISFITGEAS 50  
 ||||.::||||| ||||:| ||..|||||||||:||||||:|||:||||.  
 1 REQDQYMPIANVIRIMRKTLPSHAKISDDAKETIQECVSEYISFVTGEAN 50  
 51 DKCQREKRKTINGDDLLWAMTTLGFEDYVEPLKVYLQKVR 90  
 ::||||.|||| :|||.. ||||:||| :|| :|||  
 51 ERCQREQRKTITAEDILWAMSCLGFNDNYVDPLTVFINRYR 90

## EXHIBIT A

## CURRICULUM VITAE

JOHN JIRO HARADA

## I. BIOGRAPHICAL DATA

## A. ADDRESS

1. UNIVERSITY: Section of Plant Biology  
Division of Biological Sciences  
University of California  
One Shields Avenue  
Davis, CA 95616  
telephone: (530) 752-0673  
FAX: (530) 752-5410  
E-mail: jjharada@ucdavis.edu

2. HOME: 115 Grande Avenue  
Davis, CA 95616  
telephone: (530) 756-7042

B. BIRTHDATE: March 7, 1953

C. CITIZENSHIP: United States of America

D. MARTIAL STATUS: Married, with three children

## II. EDUCATION

## A. UNDERGRADUATE

1. DEGREE: B.S., Biochemistry, 1975  
2. INSTITUTION: University of California, Los Angeles  
3. DATES OF ATTENDANCE: October, 1970 - March, 1975  
4. RESEARCH EXPERIENCE: Undergraduate research on plant diterpene metabolism  
in the laboratory of Dr. Charles A. West, Department of Chemistry and  
Biochemistry

## B. GRADUATE

1. DEGREE: Ph.D., Biochemistry, 1981  
2. INSTITUTION: University of Washington, Seattle  
3. DATES OF ATTENDANCE: September, 1975 - December, 1980  
4. THESIS ADVISOR: Dr. David R. Morris, Department of Biochemistry  
5. PH.D. DISSERTATION: Proliferation of Polyamine-Limited Chinese Hamster  
Ovary Cells

**III. PROFESSIONAL EXPERIENCE**

A. **POSTGRADUATE RESEARCH ASSOCIATE**  
Laboratory of Dr. Robert B. Goldberg  
Department of Biology  
University of California, Los Angeles  
Los Angeles, CA 90024  
February, 1981 - August, 1984

B. **ASSISTANT PROFESSOR**  
Department of Botany  
University of California, Davis  
Davis, CA 95616  
September, 1984 - June, 1990

C. **ASSOCIATE PROFESSOR**  
Department of Botany  
University of California, Davis  
Davis, CA 95616  
July, 1990 - June, 1994

C. **PROFESSOR**  
Section of Plant Biology  
Division of Biological Sciences  
University of California, Davis  
Davis, CA 95616  
July, 1994 - present

**IV. HONORS**

President's Fellowship, University of California, 1970-71

California State Scholarship, 1970-74

Annual Fund Doctoral Fellowship, University of Washington, 1977-78

Faculty Development Award, University of California, Davis, 1988-89

**V. PROFESSIONAL ACTIVITIES****A. MEMBERSHIP IN SOCIETIES**

American Association for the Advancement of Science

American Society of Plant Physiologists

International Society for Plant Molecular Biology

University of California, Berkeley

**B. SERVICE ON REVIEW PANELS**

Ad Hoc Panel Member, NIH Biological Sciences Study Section, March, 1991

Member, Committee of Visitors to Review the NSF Developmental Biology Program, July, 1991

Member, Advisory Panel for the NSF Developmental Mechanisms Cluster, April, 1992 - March, 1997

Member, Integrative Graduate Education and Research Training Program, September, 1998

NSERC Site Visit Committee, McGill University, January, 2000

Member, Advisory Panel for the NSF Living Stocks Collection Program, December 2000

Member, Review Panel for the DOE Energy Biosciences Program, December 2001

**C. SERVICE AS REVIEWER**

**1. EDITORIAL BOARDS**

Guest Editor, *Plant Molecular Biology*, March, 1992 - March, 1997

Advisory Board, *Plant Physiology and Biochemistry*, March, 1994 - January, 2000

Co-Editor, *The Plant Cell*, July, 1996 - present

Advisory Board, *Journal of Plant Biology*, March, 1998 - present

Editorial Board, *Plant and Cell Physiology*, January 1999 - present

**2. JOURNALS**

*Archives of Biochemistry and Biophysics*

*Biochemistry*

*Development*

*Gene*

*Genetics*

*Journal of Biological Chemistry*

*Molecular and General Genetics*

*Plant Cell*

*Plant Cell Reports*

*Plant Molecular Biology*

*Plant Physiology*

*Plant Physiology and Biochemistry*

*Plant Science*

*Physiologia Plantarum*

*Proceedings of the National Academy of Sciences USA*

*Science*

**3. GRANT APPLICATIONS**

Department of Energy  
National Science Foundation  
United States Department of Agriculture - Agricultural  
Research Service  
United States Department of Agriculture - Competitive  
Research Grants Program  
United States-Israel Binational Science Foundation

**VI. TEACHING EXPERIENCE****A. TEACHING ASSISTANT**

Biochemistry Courses, University of Washington

**B. FACULTY INSTRUCTOR**

1. Lecture course in Plant Molecular Biology, Botany/Plant Biology 227, University of California, Davis, Spring, 1986 - 1996.
2. Laboratory course in Plant Molecular Biology, Botany/Plant Biology 228, University of California, Davis (co-instructor with Alan B. Bennett), Winter, 1990 - 1995.
3. Lecture course in Molecular Biology of Plant Development, Botany 125, University of California, Davis, Spring, 1995 - Spring, 1996.
4. Laboratory course in Plant Molecular Genetics, Plant Biology 198, University of California, Davis, Winter, 1997
5. Lecture course in Molecular and Cellular Biology of Plants, Plant Biology 113/113D, University of California, Davis, Spring, 1997 - present
6. Laboratory course in Plant Biotechnology Laboratory, Plant Biology 161B, University of California, Davis, Winter, 1999 - Winter 2000
7. Lecture course in Plant Growth and Development, Plant Biology 112/112D, University of California, Davis, Winter 2002 - present

**C. COURSE FOR COMMUNITY COLLEGE AND STATE UNIVERSITY INSTRUCTORS**

NSF Sponsored Workshop for the Inexperienced in Molecular Biology (Instructors: J. Christman, R. Tait, J. Harada), California State University, Sonoma, June 16-28, 1991.

**VII. UNIVERSITY SERVICE****A. GRADUATE GROUP MEMBERSHIP AND AFFILIATION WITH TRAINING PROGRAMS**

Biochemistry Graduate Group, 1985 - present

Biotechnology NIH Training Grant, 1990 - present

Cell and Developmental Biology Graduate Group, 1986 - present

Genetics Graduate Group, 1985 - present

Plant Biology Graduate Group (Formerly Botany and Plant Physiology Graduate Groups), 1985 - present

McKnight Foundation Fellowship Program: Biochemistry and Genetics of Plant-Pathogen Interactions, 1985 - 1989

Molecular and Cellular Biology NIH Training Grant, 1986 - present

Molecular Biology of the Plant Cell Program, 1987 - 1992

NSF-BBS Plant Cell Biology Training Grant (Co-Director with M.E. Etzler), 1990 - present

University of California Biotechnology Research and Education Program: Biotechnology-Goals for a Sustainable Agriculture: Mechanisms and Control of Plant and Animal Disease Using Recombinant DNA Techniques, 1985 - 1987

University of California Biotechnology Research and Education Program: Protein Targeting in Plants, 1988 - 1991

**B. ADMINISTRATIVE CHAIR POSITIONS**

1. Chair, Steering Committee for the Formation of the Section of Plant Biology, Division of Biological Sciences, 1991 - 1993
2. Co-Director, NSF Plant Cell Biology Training Program, 1990 - present
3. Chair, Committee-In-Charge of the Plant Biology Major, 1993 - 1997
4. Chair, Graduate Program in Plant Biology, 2001 - present

**VIII. LECTURES / INVITED SEMINARS**

1977 Gordon Conference on Polyamines

1979 Gordon Conference on Polyamines

1982 American Society of Plant Physiologists Meeting, Secretary's Symposium

1983 Gordon Conference on Plant Molecular Biology

Cold Spring Harbor Course on Plant Molecular Biology

Ciba-Geigy Agricultural Division, Biotechnology Research, Research Triangle Park,  
North Carolina

FMC Corporation, Agricultural Chemical Group, Princeton, New Jersey

University of Nebraska, Lincoln

1984 University of California, Davis

University of Wisconsin, Madison

Purdue University, West Lafayette, Indiana

University of California, Riverside

University of Illinois, Champaign-Urbana

Tokyo Meeting of Plant Biotechnology, Tokyo, Japan

International Symposium on Genetic Manipulation in Crops, Beijing, China

1985 American Society for Plant Physiologists Western Section Meeting, Plant Molecular  
Genetics Symposium

1987 Monsanto Company, St. Louis, Missouri

University of California, Los Angeles

1988 Purdue University, West Lafayette, Indiana

UCLA Symposium: The Molecular Biology of Plant Development, Steamboat Springs,  
Colorado (session chair and speaker)

American Society for Plant Physiologists Meeting, Gene Expression Session, Reno,  
Nevada

Washington State University, Pullman

1989 Carnegie Institution of Washington, Stanford, California

Workshop on Embryogenic Plant Systems in Developmental Genetics and  
Biotechnology, Ottawa, Ontario, Canada

Fifth Annual Crucifer Genetics Workshop, Davis, California

	American Society of Plant Physiologists Meeting, Symposium, Toronto, Ontario, Canada
	Horticultural Biotechnology Symposium (session chair), Davis, California
	Kobe University, Kobe, Japan
	Plantech Research Institute, Yokohama-City, Japan
	US-Japan Cooperative Science Program Meeting, Tokyo, Japan
1990	Monsanto Company, St. Louis, Missouri
	Tissue Culture Association Meeting, Houston, Texas
	US-Japan Cooperative Science Program Meeting, Davis, California
1991	Fourteenth Annual Symposium in Plant Physiology, Molecular Approaches to Compartmentation and Metabolic Regulation, Riverside, California
	Keystone Symposia on Molecular and Cellular Biology, The Genetic Dissection of Plant Cell Processes, Keystone, Colorado
	Twenty-seventh National Institute for Basic Biology Conference, Plant Organelle Proteins; Biosynthesis, Targeting, and Assembly, Okazaki, Japan
	Plantech Research Institute, Yokohama-City, Japan
	Nagoya University, Nagoya, Japan
1992	Crop Molecular Biology and Biotechnology Workshop, Banff, Alberta, Canada
1993	Sonoma State University, Rohnert Park, California
	XV International Botanical Congress, Tokyo, Japan (Session chair and speaker in two sessions)
1994	Monsanto Company, St. Louis, Missouri
	University of Wisconsin, Madison
	Fourth International Congress of Plant Molecular Biology, Amsterdam, The Netherlands
	Texas A&M University, College Station
	University of Arizona, Tucson
	Purdue University, West Lafayette, Indiana
	Jacques Monod Conference, "Signal Transduction during Plant Embryogenesis", Aussois, France

1996	University of California, Los Angeles University of Alberta, Edmonton, CANADA Calgene, Inc., Davis, California FASEB Conference, "Plant Developmental Genetics", Saxon River, Vermont 50th Anniversary of the Korean Association of Biological Sciences, Seoul, KOREA
1997	Hokkaido University, JAPAN Thirty-ninth National Institute of Basic Biology Conference, "Dynamic Aspects of Seed Maturation and Germination", Okazaki, JAPAN Information Processing Systems in Plants Their Evolution and Function (session chair) Davis, California Sonoma State University, Rohnert Park, California University of Minnesota, St. Paul, Minnesota
1998	Seed Physiology Meeting, Davis, California The 7th NIAR/COE International Symposium, "Transcription Factors Controlling Plant Development", Tokyo, JAPAN National Institute of Basic Biology Conference, Okazaki, JAPAN Sacramento State University, California University of Vienna Biocenter, AUSTRIA Fifth Plant Embryogenesis Workshop, Barcelona, SPAIN
1999	International Botanical Congress XVI, St. Louis, Missouri
2000	World Congress on In Vitro Biology, San Diego, California Eleventh International Arabidopsis Conference, Madison, Wisconsin FASEB Summer Research Conference on Mechanisms in Plant Development, Saxon River, Vermont The 8th International Symposium on Plant Seeds/5th Gatersleben Research Conference, Gatersleben, GERMANY
2001	DNA Plant Technology, Oakland, CA University of Washington, Seattle

Society for Developmental Biology, Northwest Regional Meeting, Friday Harbor,  
Washington

University of Kentucky, Lexington

## IX. RESEARCH SUPPORT

### A. J.J. HARADA AS PI

NSF: DCB-8518182; Developmental Regulation of Germination-Induced Genes; 4/86 - 4/89; \$202,900 (Total)

USDA: 86-CRCR-1-2172; Molecular Aspects of Disease in Higher Plants (co-PI: D. Gilchrist); 9/86 - 9/88; \$100,000 (Total)

USDA: 88-37262-3542; Molecular Analysis of Protein Targeting to Glyoxysomes; 7/88 - 7/90; \$120,000 (Total)

NSF: DCB-8819315; Gene Regulation in Glyoxysome Biogenesis; 4/89 - 4/92; \$231,460 (Total)

Plantech Research Institute; Gene Regulation during Embryogeny; 4/90 - 10/91; \$50,647 (Total)

USDA: 90-37261-5515; Peroxisome Biogenesis in Higher Plants; 9/90 - 8/93; \$171,000 (Total)

NSF: IBN-9118120; Gene Regulation in Glyoxysome Biogenesis; 4/92 - 3/94; \$200,000 (Total)

NSF: IBN-9317526; Regulation of Glyoxysome Formation during Higher Plant Development; 4/94 - 3/98; \$360,000 (Total)

DOE: DE-FG03-94ER20139; Regulation of Embryonic Development in Higher Plants; 5/94 - 5/97; \$276,069 (Total)

DOE: DE-FG03-94ER20139; Regulation of Embryonic Development in Higher Plants; J. Harada; 5/97 - 5/00; 288,000 (Total)

Ceres Inc.; Regulators of Embryo and Seed Development, 10/98 - 10/03; \$190,000 (Annual).

DOE: DE-FG03-94ER20139; Regulation of Embryonic Development in Higher Plants; J. Harada; 5/00 - 5/03; \$315,000 (Total)

### B. J.J. HARADA AS CO-PI OR COOPERATOR

McKnight Foundation; Biochemistry and Genetics of Plant-Pathogen Interactions (PIs: T. Kosuge, G. Bruening, D. Gilchrist); 9/83 - 8/86 & 9/86 - 8/89; \$1,500,000 (Total)

U.C. Biotechnology Research and Training Program; Biotechnology-Goals for a Sustainable Agriculture: Mechanisms and Control of Plant and Animal Disease Using Recombinant DNA Techniques (PI: R. Michelmore); 7/85 - 6/87; \$230,000 (Total)

U.C. Biotechnology Research and Education Program; Molecular Basis of Protein Targeting to Subcellular Compartments of Plant Cells (PI: A. Bennett); 7/87 - 6/90; \$300,000 (Total)

NSF U.S.-Japan Cooperative Science Program; Plant Cell Bioprocess Engineering for Production of Secondary Metabolites (P.I.: D. Ryu); 4/89 - 3/91; \$38,000 (Total)

NSF: DIR-9014274 (Training Grant); Plant Cell Biology Training Program (Co-Directors: M. Etzler and J. Harada); 9/90 - 9/95; \$2,520,038 (Total)

NSF: DIR 8920216; Planning and Managing Center of Engineering Plants for Resistance Against Pathogens (PI: G Bruening); 2/91 - 1/96; ~\$3,500,000

NSF: BIR-9414106; Plant Cell Biology Training Program (Co-PIs: M. Etzler and J. Harada); 7/95-6/00; \$938,532

#### X. PUBLICATIONS

1. Coates, R.M., Conradi, R.A., Ley, D.A., Akeson, A., Harada, J., Lee, S.C., and West, C.A. (1976). Enzymatic cyclization of (R,S)-14,15-oxidogeranylgeranyl pyrophosphate to 3 $\alpha$ - and 3 $\beta$ -hydroxykaurene. *J. Am. Chem. Soc.* 98: 4659-4661.
2. Jorstad, C.M., Harada, J.J., and Morris, D.R. (1980). Structural specificity of the spermidine requirement of an *Escherichia coli* auxotroph. *J. Bacteriol.* 141: 456-463.
3. Morris, D.R. and Harada, J.J. (1980). The participation of polyamines in the proliferation of bacterial and animal cells. In: *Polyamines in Biomedical Research*, J. Gaugas, ed., John Wiley and Sons, Ltd., London, pp. 1-16.
4. Harada, J.J. (1981). Effects of polyamine limitation on the growth of animal cells. Ph.D. Thesis, University of Washington, Seattle, Washington.
5. Harada, J.J., Porter, C.W., and Morris, D.R. (1981). Induction of polyamine limitation in Chinese hamster ovary cells by  $\alpha$ -methyl-ornithine. *J. Cell. Physiol.* 107: 413-426.
6. Harada, J.J. and Morris, D.R. (1981). Cell cycle parameters of Chinese hamster ovary cells during exponential, polyamine-limited growth. *Molec. Cell. Biol.* 1: 594-599.
7. Young, E.T., Menard, R.C., and Harada, J. (1981). Monocistronic and polycistronic bacteriophage T4 gene 23 messages. *J. Virol.* 40: 790-799.
8. Goldberg, R.B., Fischer, R.B., Harada, J.J., Jofuku, D., and Okamuro, J.K. (1983). Organization of soybean seed protein genes and their flanking regions. In: *Structure and Function of Plant Genomes*, O. Cifelli and L. Dure III, eds., Plenum Publishing Corporation, New York, pp. 37-45.

9. Bruening, G., Harada, J., Kosuge, T., and Hollaender, A. eds. (1987). *Tailoring Genes for Crop Improvement: an Agricultural Perspective*, Plenum Press, New York.
10. Barker, S.J., Harada, J.J., and Goldberg, R.B. (1988). Cellular localization of soybean storage protein mRNA in transformed tobacco seeds. *Proc. Natl. Acad. Sci. USA* 85: 458-462.
11. Harada, J.J., Baden, C.S., and Comai, L. (1988). Spatially regulated genes expressed during seed germination and postgerminative development are activated during embryogeny. *Mol. Gen. Genet.* 212: 466-473.
12. Harada, J.J., Dietrich, R.A., Comai, L., and Baden, C.S. (1988). Regulation of gene expression during seed germination and postgerminative development. In: *Plant Gene Research, Volume 5, Temporal and Spatial Regulation of Plant Genes*, D.P.S. Verma and R.B. Goldberg, eds., Springer-Verlag, New York, pp. 26-39.
13. Dietrich, R.A., Maslyar, D.J., Heupel, R.C., and Harada, J.J. (1989). Spatial patterns of gene expression in *Brassica napus* seedlings: Identification of a cortex-specific gene and localization of mRNAs encoding isocitrate lyase and a polypeptide homologous to proteinases. *Plant Cell* 1: 73-80.
14. Comai, L., Baden, C.S., and Harada, J.J. (1989). Deduced sequence of a malate synthase polypeptide encoded by a subclass of the gene family. *J. Biol. Chem.* 264: 2778-2782.
15. Comai, L., Dietrich, R.A., Maslyar, D.J., Baden, C.S., and Harada, J.J. (1989). Coordinate expression of transcriptionally regulated isocitrate lyase and malate synthase genes in *Brassica napus* L. *Plant Cell* 1: 293-300.
16. Harada, J.J., DeLisle, A.J., Baden, C.S., and Crouch, M.L. (1989). Unusual sequence of an abscisic acid-inducible mRNA which accumulates late in *Brassica napus* seed development. *Plant Mol. Biol.* 12: 395-401.
17. Harada, J.J., Barker, S.J., and Goldberg, R.B. (1989). Soybean  $\beta$ -conglycinin genes are clustered in several DNA regions and are regulated by transcriptional and posttranscriptional processes. *Plant Cell* 1: 415-425.
18. Gilchrist, D.G. and Harada, J.J. (1989). Mode and physiological consequence of AAL-toxin interaction with the *asc* locus in tomato. In: *Phytotoxins and Plant Pathogenesis*, NATO ASI Series, Vol. H27, A. Graniti et al., eds., Springer-Verlag, Berlin, pp. 113-121.
19. Dure, L. III., Crouch, M., Harada, J., Ho, T.-H.D., Mundy, J., Quatrano, R., Thomas, T., and Sung, Z.R. (1989). Common amino acid sequence domains among the LEA proteins of higher plants. *Plant Mol. Biol.* 12: 475-486.
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22. Ettinger, W.F. and Harada, J.J. (1990). Translational or posttranslational processes affect differentially the accumulation of isocitrate lyase and malate synthase proteins and enzyme activities in embryos and seedlings of *Brassica napus*. *Arch. Biochem. Biophys.* 281: 139-143.
23. McGrath, J., Mitchell, Quiros, C.F., Harada, J.J., and Landry, B.S. (1990). Identification of *Brassica oleracea* monosomic alien chromosome addition lines with molecular markers reveals extensive gene duplication. *Mol. Gen. Genet.* 223: 198-204.
24. Harada, J.J. (1990). Summary: Biotic Stress. In: *Horticultural Biotechnology*, A.B. Bennett and S.D. O'Neill, eds., Wiley-Liss, Inc., New York, pp. 261-262.
25. Olsen, L.J. and Harada, J.J. (1991). Biogenesis of peroxisomes in higher plants. In: *Molecular Approaches to Compartmentation and Metabolic Regulation*, A.H.C. Huang and Lincoln Taiz, eds., The American Society of Plant Physiologists, Rockville, MD, pp. 129-137.
26. Landry, B.S., Hubert, N., Etoh, T., Harada, J.J., and Lincoln, S.E. (1991). A genetic map of *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. *Genome* 34: 543-552.
27. Comai, L., Matsudaira, K.L., Heupel, R.C., Dietrich, R.A., and Harada, J.J. (1992). Expression of a *Brassica napus* malate synthase gene in transgenic tomato plants during the transition from late embryogeny to germination. *Plant Physiol.* 98: 53-61.
28. Dietrich, R.A., Radke, S.E., and Harada, J.J. (1992). Downstream DNA sequences are required to activate a gene expressed in the root cortex of embryos and seedlings. *Plant Cell* 4: 1371-1382.
29. Zhang, J.Z., Gomez-Pedrozo, M., Baden, C.S., and Harada, J.J. (1993). Two classes of isocitrate lyase genes are expressed during late embryogeny and postgermination in *Brassica napus* L. *Mol. Gen. Genet.* 238: 177-184.
30. Olsen, L.J., Ettinger, W.F., Damsz, B., Matsudaira, K.L., Webb, M.A., and Harada, J.J. (1993). Targeting of glyoxysomal proteins to peroxisomes in leaves and roots of a higher plant. *Plant Cell* 5: 941-952.
31. West, M.A.L. and Harada, J.J. (1993). Embryogenesis in higher plants: an overview. *Plant Cell* 5: 1361-1369.
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33. Zhang, J.Z. and Harada, J.J. (1994). Gene Expression in Plant Cells. In: *Advances in Plant Biotechnology: Production of Secondary Metabolites*, S. Furusaki and D. Ryu, eds., Elsevier Science Publishers B.V., Amsterdam, pp. 1-10.
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36. Olsen, L.J. and Harada, J.J. (1995). Peroxisomes and their assembly in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46: 123-146.
37. Zhang, J.Z., Santes, C.M., Engel, M.L., Gasser, C.S., and Harada, J.J. (1996). DNA sequences that activate isocitrate lyase gene expression during late embryogenesis and during postgerminative growth. *Plant Physiol.* 110: 1069-1079.
38. Brickner, D.G., Harada, J.J., and Olsen, L.J. (1997). Protein transport into higher plant peroxisomes. *In vitro* import assay provides evidence for receptor involvement. *Plant Physiol.* 113: 1213-1221.
39. Harada, J.J. (1997). Seed maturation and control of germination. In: *Advances in Cellular and Molecular Biology of Plants, Volume 4, Cellular and Molecular Biology of Seed Development*. B.A. Larkins and I.K. Vasil, eds., Kluwer Academic Publishers, Dordrecht, The Netherlands, 545-592.
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42. Kragler, F., Lametschwandtner, G., Christmann, J., Hartig, A., and Harada, J.J. (1998). Identification and analysis of the plant peroxisomal targeting signal 1 receptor NtPEX5. *Proc. Natl. Acad. Sci. USA* 95: 13336-13341.
43. Harada, J.J., Lotan, T., Fischer, R.L., and Goldberg, R.B. (1998). ...response: Embryos without sex. *Trends Plant Sci.* 3: 452-453.
44. Harada, J.J. (1999). Signaling in plant embryogenesis. *Curr. Opin. Plant Biol.* 2: 23-27.
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50. Apuya, N. R., Yadegari, R., Fischer, R.L., Harada, J.J., Zimmerman, J.L., and Goldberg, R.B. (2001). The *Arabidopsis* embryo mutant *schlepperless* has a defect in the chaperonin-60alpha gene. *Plant Phys.* 126:717-730.
51. Stone, S.L., Kwong, L.W., Matsudaira Yee, K., Pelletier, J., Lepiniec, L., Fischer, R.L., Goldberg, R.B., and Harada, John J. (2001). *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. *Proc. Natl. Acad. Sci. USA*, 98: 11806-11811.
52. Kinoshita, T., Harada, J.J., Goldberg, R.B., and Fischer, R.L. (2001). Polycomb repression of flowering during early plant development. *Proc. Natl. Acad. Sci. USA* 98: 14156-14161.
53. Weterings, K., Apuya, N.R., Bi, Y., Fischer, R.L., Harada, J.J., and Goldberg, R.B. (2001). Regional localization of suspensor mRNAs during early embryo development. *Plant Cell*, 13:2409-2425.

#### XI. PATENTS

1. Harada, J.J. and Zhang, J.Z. Plant promoter sequences useful for gene expression in seeds and seedlings. U.S. Patent No. 5,689,040
2. Fischer, R.L., Ohad, N., Kiyosue, T., Yadegari, R., Margossian, L., Harada, J., and Goldberg, R.B., Nucleic acids that control endosperm development in plants, US Patent No. 6,229,064
3. Harada, J.J., Lotan, T., Ohto, M., Goldberg, R.B., and Fischer, R.L., *LEAFY COTYLEDON1* genes and methods of modulating embryo development in transgenic plants. US Patent No. 6,235,975

## XII. STUDENTS AND POSTDOCTORAL ASSOCIATES TRAINED

### A. POSTDOCTORAL ASSOCIATES

*Dr. Barney Ward*, 10/85-6/88; current position, Research Associate, Department of Plant Pathology, University of California, Davis

*Dr. William F. Ettinger*, 10/87-2/90; current position, Associate Professor, Department of Biology, Gonzaga University, Spokane, WA

*Dr. Mariza Gomez-Pedroso*, 5/88-10/88; current position, unknown

*Dr. Edward J. Newbigin*, 2/89-3/90 (Joint postdoc with M.E. Etzler); current position, Laboratory Director, School of Botany, University of Melbourne

*Dr. Laura J. Olsen*, 4/90-8/93; current position, Associate Professor, Department of Biology, University of Michigan

*Dr. Marilyn A.L. West*, 1/92-4/95; current position, Lab Manager, Department of Vegetable Crops, University of California, Davis

*Dr. Christina Santes Valera*, 1/94-12/96; current position, science teacher, Davis, California

*Dr. Tamar Lotan*, 4/95-7/98; current position, establishing biotechnology company in Israel

*Dr. Kazutoshi Yamagishi*, 4/95-5/98; current position, Postdoctoral Fellow, RIKEN, Japan

*Dr. Masa-aki Ohto*, 5/95-2/96 & 5/01-present; past position, Assistant Professor, Department of Plant Biochemistry, National Institute of Basic Biology, Okazaki, Japan

*Dr. Luis Perez Grau*; 3/00-6/01; current position, Scientist, Simplot Corporation, Boise, ID

*Dr. Sandra L. Stone*, 5/01-present

### B. GRADUATE STUDENTS

*Lucio Comai*, Ph.D., Biochemistry, December, 1990. Thesis: Regulation of malate synthase gene expression during embryogeny and postgermination in *Brassica napus*. Damon-Ryun Postdoctoral Fellow with R. Tjian, University of California, Berkeley. Current position, Associate Professor, Department of Microbiology, University of Southern California

*Robert A. Dietrich*, Ph.D. Genetics, September, 1991, McKnight Graduate Fellow. Thesis: Regulation of a *Brassica napus* gene expressed during embryogenesis and postgerminative growth. Current Position: Scientist, Syngentia, Inc., Research Triangle

Park, NC

*James Z. Zhang*, Ph.D. Genetics, March, 1993. Thesis: Developmental Regulation of Isocitrate Lyase Gene Expression in *Brassica napus* L. Current Position: Scientist, Mendel Biotechnology, Hayward, CA

*Debbie L. Laudencia-Chingcuanco*, Ph.D. Genetics, December 1994, UC Dissertation Fellow. Thesis: Functional Analysis of the Regulatory DNA Sequences of a Malate Synthase Gene from *Brassica napus*. Current position, NSF Postdoctoral Fellow with S. Hake, University of California, Berkeley.

*Elizabeth A. Wasson*, M.S. Plant Biology, July, 1994, Plant Cell Biology Graduate Fellow.

*Martin P. Doyle*, M.S. Plant Biology, August, 1995, Plant Cell Biology Graduate Fellow.

*Minsung Kim*, October, 1996 - 1998, Genetics Graduate Group Ph.D. student.

*Raymond W. Kwong*, September, 1998 - present, Plant Biology Graduate Group Ph.D. Student

*Hye-seung Lee*, September, 1998 - present, Plant Biology Graduate Group Ph.D. Student

C. UNDERGRADUATE STUDENTS (NAME, YEARS INVOLVED IN RESEARCH, CURRENT POSITION)

*Davila, Alvaro*, 1985, Unknown

*Goldman, Polly*, 1986, Graduate student, UCSC

*Maslyar, Daniel*, 1987 - 1990, Medical student, UCSF

*Nguyenle, Thuylinh*, 1989 - 1990, Unknown

*Holst, Eric*, 1990, Graduate student, Duke University

*Morales, Consuelo*, 1990 - 1995, Employed

*Danao, Jay*, 1990 - 1995, Employed

*Li, Morris*, 1991, Medical student, Loyola University

*Fernandez, Lewis*, 1991, Graduate school

*Chan, Victor*, 1991 - 1992, Technician, Sandoz

*Pham, Nhu*, 1991, Unknown

*Hartt, Gregory*, 1992 - 1993, M.D./Ph.D. student, Ohio State University

*Tran, Minh*, 1992, Pharmacy student, Creighton University

*Willard, Traci*, 1992, Employed

*Renouf, Lynette*, 1992, Unknown

*Culberson, Carla*, 1992, Unknown

*Hague, Carolyn*, 1993, Employed

*Engel, Michelle*, 1993 - 1994, Graduate student, UCLA

*Togioka, Patti*, 1993 - 1994, Employed

*Dung, Hoa*, 1993 - 1994, Employed

*Lo, Russell*, 1993 - 1995, Ph.D. student, University of Washington

*Heraux, Jonathan*, 1994, Student, University of Hawaii

*Gabaldon, Amapro*, 1994, Student, University of Madrid

*Anthony Phengrasamy, 1995, Pharmacy student, UCSF  
Wortley, Sara, 1995 - 1997, unknown  
Swirsding, Kendra 1995 - 1997, Technician  
Leong, Mary , 1995 - 1997, Optometry School  
Chao, Kim 1995- 1997, Unknown  
Lee, Vong 1995 - 1996, Unknown  
Baxter, Kim, 1996 - 1997, Graduate Student  
Kwong, Raymond, 1996 - 1997, Graduate Student, UCD  
Jennifer Lee, 1997, 1998 - 1999, Medical Student, UCSF  
Linda Kwong, 1997- 1999, Technician  
Michael Gonzales, 1997 - 1999, Graduate Student, University of Wisconsin, Madison  
Yue-yun To, 1998, unknown  
Tereza Kolesnikov, 1998 - 1999, Technician, Stanford University  
Kyle Mizuno, 1998 - 1999, Biochemist  
Diana Lee, 1999 - 2000, Student  
Christy Ferlatte, 2000-2001, Student  
Stephanie Paula, 2001-present, Student  
Abeba Kiros, 2001-present, Student*